

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s): Semple et al.

Application No.: 09/654,373

Filed: 9/1/2000

Title: Novel Small Multilamellar Vesicle Structures

Attorney Docket No.: INEX.P-007

Customer No.: 021121

Group Art Unit: 1651

Examiner: David Naff

Confirmation No: 5857

**DECLARATION OF MICHAEL J. HOPE UNDER RULE 132**

I, the undersigned declare as follows:

1. I am a named inventor of the above-captioned application. As such, I am familiar with the application, including the claims thereof.
2. In the application as filed, the polyamine DOGS (dioctadecylamidoglycylspermine) is mentioned as an example of an ionizable lipid. This reference to DOGS is an error.
3. DOGS is a polyamine, which includes four positively charged amines. These amines each have a separate pKa value, and these values have been reported to be 10.5, 9.5, 8.4 and 5.5. Using the Henderson-Hasselbach equation,

$$\text{pH} = \text{pKa} + \log\{ [A]/[HA] \}$$

which relates the extent of ionization of an acid HA to its deprotonated form A to the pKa value and the actual pH, one can calculate the extent to which each amine is deprotonated at pH 7.5. In this case, A is actually charge neutral, and the HA species has a positive charge.

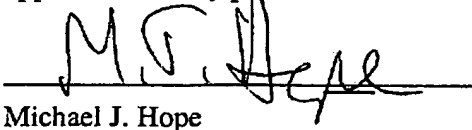
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amine pKa	ratio of neutral to positive charges	excess charge type
5.5	100	neutral
8.4	.126	positive
9.5	0.01	positive
10.5	0.001	positive

Thus, at pH 7.5, nearly every molecule (99 out of 100) of DOGS will have two positive charges, and of these more than 80 will have three positive charges. In contrast, DODAP, which has a pKa of 6.7 has a ratio of neutral to positive at pH 7.5 of 6.3 and more neutral molecules than positively charged molecules at this pH. DODMA has essentially the same pKa as DODAP.

4. An important feature of the invention of this application is the fact that a pH change to near physiological pH, for example to around 7.5, results in the release of complexed oligonucleotides from the exterior of the lipid-nucleic acid particle. This would not occur to any significant extent if DOGS was used as the cationic lipid, because of the large number of positive charges that would remain. Accordingly, DOGS would not work in the present invention, and inclusion of DOGS in the original application was a readily recognizable error which is being corrected by deletion of the reference to DOGS in the application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

  
Michael J. Hope

Dec. 12, 2003.  
Date

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